CHEST Online Supplement

Secondhand Smoking Is Associated With Vascular Inflammation

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Methods

Immunofluorescence for Protein Expression

Endothelial cells were recovered by centrifugation and fixed with 2% formaldehyde in PBS for 10 min, washed twice with PBS, transferred to poly-L-lysine coated slides (Sigma, St. Louis, MO), and air dried at 37°C. The slides were stored at -80°C until analyzed. Each endothelial harvesting yielded 2348±1247 endothelial cells (mean±SD; range 840-5536).

Endothelial cells were permeabilized in PBS/0.5% Triton X-100. Non-specific sites were blocked with PBS-5% donkey serum. Endothelial cells were incubated with monoclonal antibodies against endothelial nitric oxide synthase (eNOS), (Becton Dickinson Transduction Laboratories, San Jose, CA), phosphorylated eNOS at serine1177 (P-eNOS), nitrotyrosine (Upstate Biotechnology, Chicago, IL) and nuclear factor kappa B (NFκB) (Novus Biologicals, Littleton, CO), and followed by Cy3-conjugated donkey anti-mouse antibodies (Jackson ImmunoResearch Laboratories, West Grove, PA). Appropriate negative control slides were generated using preimmune IgG. Polyclonal anti-von Willebrand factor antibodies (DAKO, Glostrup, Denmark) were then used, followed by FITC-conjugated secondary antibodies. Nuclei were stained with diaminophenylindole (DAPI) (Molecular Probes, Carlsbad, CA). Between experiments variability was standardized using reference slides of human umbilical venous endothelial cells (HUVEC) obtained from the same culture dish. Slides from study participants were stained concurrently with one slide of HUVEC.

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Endothelial cells were analyzed with a fluorescent microscope under identical conditions (Nikon Eclipse E600, Melville, NY), and were captured by digital camera (Q Imaging Retiga EXi, Surrey, BC, Canada). The reader was blinded to subjects' identity. Nuclear and von Willebrand factor staining identified endothelial cells. Slides were systematically read left to right and top to bottom. Twenty-five consecutive endothelial cells were analyzed from each slide. The number of positive (bright) intracellular pixels was quantified using commercially available software (Image J), and normalized to reference HUVEC slides to calculate pixel ratios (arbitrary units).[1-4]

References:

1. Onat D, Jelic S, Schmidt AM, et al. Vascular Endothelial Sampling and Analysis of Gene Transcripts: a New Quantitative Approach to Monitor Vascular Inflammation. *J Appl Physiol* 2007;103(5):1873-1878.

2. Jelic S, Padeletti M, Kawut SM, et al. Inflammation, oxidative stress, and repair capacity of the vascular endothelium in obstructive sleep apnea. *Circulation* 2008;117(17):2270-2278.

3. Jelic S, Lederer D, Adams T, et al. Endothelial Repair Capacity and Apoptosis are Inversely Related in Obstructive Sleep Apnea. *Vasc Health Risk Manag* 2009;5:909-920.

4. Colombo PC, Onat D, Harxhi A, et al. Peripheral venous congestion causes inflammation, neurohormonal, and endothelial cell activation. *Eur Heart J* 2013;35(7):448-454.

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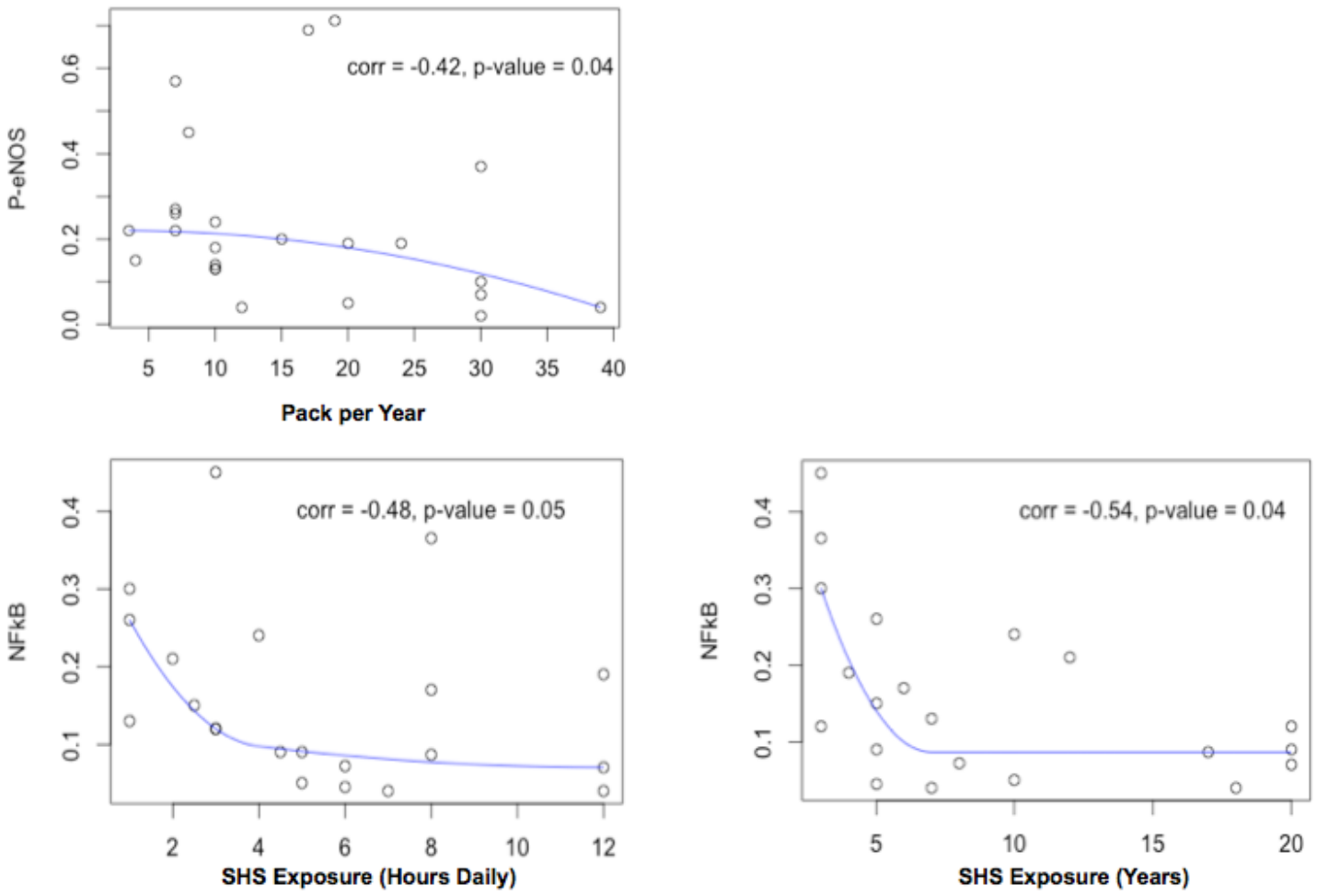
e-Figure 1. Significant Relationship between Markers of Endothelial Reactivity and Inflammation and Intensity of Cigarette Smoke Exposure.



Expression of P-eNOS (Phosphorylated Endothelial Nitric Oxide Synthase) in harvested venous endothelial cells was correlated inversely with pack-years of exposure to cigarette smoke in active smokers. Correlation between expression of NFκB (Nuclear Factor kappa B) and daily exposure to secondhand smoke (SHS) was borderline significant (p=0.05) whereas expression of NFκB correlated inversely with years of exposure to SHS in passive smokers. This trend was most pronounced in passive smokers who were exposed to SHS for longer than 15 years.

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